利用莖頂組織超低溫冷凍處理法去除甘藷病毒之研究

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摘要

本研究試驗材料為甘藷 [*Impomoea batatas* (L) Lam.]栽培種「台農 57 號」、「台農 66 號」與野生種「WT248」、「WT427」分別感染甘藷羽狀斑駁病毒(SPFMV)、甘藷病毒 G (SPVG)與甘藷黃矮病毒(SPCSV)，取病毒株莖頂組織為材料，以藻膠包埋玻璃質化法進行超低溫冷凍保存，於回復生長培養基(即 MS 基本鹽類)添加 1 μM NAA, 0.5 μM BA, 0.5 μM Kinetin,30 g L^{-1} 蔗糖及 9.4 g L^{-1} Difco agar。結果顯示，「台農 66 號」及「WT427」可獲得植株成活率最佳 13.1–15.2%，而「台農 57 號」僅獲得 2.2%；若以 1 μM IAA 替代 NAA，並且將 BA 濃度提高 1 倍，則「台農 57 號」的植株成活率提高為 44.6%，但野生種兩品系的植株成活率降低為 0–11.1%；以感染病毒之甘藷進行超低溫冷凍處理法去除病毒的試驗顯示，栽培種「台農 57 號」、「台農 66 號」及野生種「WT248」之再生株以 RT-PCR 均未測到上述三種病毒，去病毒率為 100%，野生種「WT427」的 9 個再生株中，5 個測到甘藷病毒 G，去病毒率為 44.4%。

關鍵詞：甘藷、超低溫冷凍保存、病毒。

Effect of Cryotherapy to Shoot Tips Culture on Elimination of Sweet Potato Virus

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ABSTRACT

Sweet potato [*Ipomoea batatas* (L) Lam.] cultivars/lines, 'Tainung No. 57 (TNG57)', 'Tainung No. 66 (TNG66)', 'WT248' and 'WT427' infected with *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus G* (SPVG) and *Sweet potato chlorotic stunt virus* (SPCSV) were cultivated at Wufeng District, Taichung City, for the field experiment. The treatment of cryotherapy was applied to the shoot tips of infected plants for preservation. The preserved shoot tips were put into the survival medium which consisted of MS (Murashige and Skoog) basic salted medium and supplemented with 1 μM NAA, 0.5 μM BA, 0.5 μM Kinetin, 30 g L^{-1} sucrose, and 9.4 g L^{-1} Difco agar. The shoot regeneration rates were 13.1–15.2% from *in vitro*-grown sweet potato of 'TNG66' or 'WT427', and was 2.2% for 'TNG57'. In the case of 1 μM IAA replaced with NAA and concentration of BA was increased twice in the survival medium, the shoot regeneration rate enhanced to 44.6% for 'TNG57', but decreased to 0–11.1% for 2 wild types of sweet potato. By multiplex RT-PCR, SPFMV, SPVG and SPCSV were undetectable in all regenerated plantlets of 'TNG 57', 'TNG 66' and 'WT248' (100% virus eradication); however, SPVG was detected in 5 out of 9 regenerated plantlets of 'WT427'.

Key words: Sweet potato, Cryopreservation, Virus.

前言

感染甘藷的病毒種類相當多，超過 9 科 30 種(Clark *et al.* 2012)，臺灣有紀錄危害甘藷的病毒有甘藷羽狀斑駁病毒(*Sweet potato feathery mottle virus;* SPFMV) (Yang 1972)、甘藷潛隱病毒(*Sweet potato latent virus;* SPLV) (Liao *et al.* 1979a, b)、甘藷病毒 G (*Sweet potato virus* G; SPVG) (Wang *et al.* 2013)、甘藷病毒 Y [*Sweet potato virus* Y; SPVY, 又稱甘藷病毒 2 (*Sweet potato virus* 2, SPV2)]、甘藷黃萎病毒(*Sweet potato yellow dwarf virus;* SPYDV)、甘藷捲葉病毒(*Sweet potato leaf curl virus;* SPLCV) (Chung *et al.* 1985, 1986, Green *et al.* 1989)和甘藷黃矮病毒(*Sweet potato chlorotic stunt virus*; SPCSV) (Cheng *et al.* 2012)等 7 種。國外報導感染甘藷的病毒中，以 SPFMV 和 SPCSV 最為常見(Loebenstein *et al.* 2003, 2009, Tairo *et al.* 2005)，二病毒單獨感染對產量影響不大，但若複合感染協力作用造成嚴重的甘藷病毒病(Sweet potato virus disease; SPVD) (Schaefers and Terry 1976)，大幅降低塊根產量(Karyeija *et al.* 2000, Mukasa *et al.* 2006)。臺灣感染甘藷的 7 種病毒中以 SPFMV 最為普遍，若與 SPLV 複合感染，使「台農 57 號」減產 24.5%，「台農 63 號」減產 35.6%，明顯減少甘藷產量(Chung *et al.* 1981)。

Brison *et al.* (1997)以超低溫冷凍法進行梅樹種原保存，可獲得 50%的健康株，而傳統切取生長點去除病毒的技術僅可獲得 20%的健康株；根據 Helliot *et al.* (2002)指出香蕉利用傳統的切取生長點去病毒方法，無法去除 CMV (*Cucumber mosaic virus*)病毒，若以超低溫冷凍法可獲得無 CMV 的植株達 2%；Wang and Valkonen (2008)利用藻膠包埋甘藷莖頂組織，成功去除 SPFMV 和 SPCSV2 種病毒，二位學者亦利用組織化學免疫定位，發現不管單獨或複合感染，SPFMV 位於第 4、5 葉原體，SPCSV 只存在於第 5 葉原體，內層第 3、2、1 葉原體與頂端分生組織無病毒存在。以莖頂組織為培植體進行藻膠包埋之液態氮處理時，外圍細胞會凍死，存活的為內層之未分化葉原體與頂端分生組織，誘導再生後可以得到去病毒的小苗。

本研究旨在以甘藷栽培種「台農 57 號」、「台農 66 號」與野生種品系「WT248」、「WT427」等 4 品種(系)病毒株之莖頂組織進行藻膠包埋超低溫冷凍法處理，據以探討此一方法去除病毒病的效率。

一、供試材料及方法

供試材料為截取甘藷「台農 57 號」、「台農 66 號」、「WT248」及「WT427」等 4 品種(系)同時感染甘藷羽狀斑駁病毒(SPFMV)、甘藷病毒 G (SPVG)與甘藷黃矮病毒(SPCSV)的病毒株莖段，經 1%次氯酸鈉溶液振盪 10 min，無菌水清洗 3–4 次後，將培植體切成單節置於含 MS 基本鹽類並添加 30 g L^{-1} 蔗糖及 9.4 g L^{-1} Difco agar 及 pH 5.7 ± 0.1 的培養基，其培養環境為光照 16 h，溫度 25℃。經 30 d 生長後，切取莖頂 1.0 mm 大小，置於藻膠培養液[含 2% (w/v) Na–alginate 及 30 g L^{-1} 蔗糖，但不含 Ca^{2+}的 MS 基本鹽類培養液]。以滴管將莖頂與藻膠培養液一同滴入包埋培養液(MS 基本培養液、0.4 M 蔗糖及 0.1 M $CaCl_2$)，並於 25℃中靜置 30 min，待其形成直徑約 0.5 cm 之球形，再將藻膠球培養於含有 30 g L^{-1} 蔗糖之 MS 基本鹽類液體培養基，每錐形瓶含有 10 粒藻膠球及 50 mL 的培養基。以震盪速率為 90 rpm 培養 1 d，再移至含有 0.3 M 蔗糖之 MS 基本鹽類液體培養基培養 16 h。將上述處理的藻膠球置於 LS 溶液(2 M 甘油 + 1.6 M 蔗糖，pH 5.8, Hirai and Sakai 2003)，處理 300 min，將 LS 溶液吸出，置入 PVS2 溶液(標準配方為 30% w/v 甘油 + 15% w/v 乙二醇 + 15% w/v 二甲基亞碸(DMSO) + 0.4 M 蔗糖， Sakai *et al.* 1990)的錐形瓶，每瓶含有 10 粒藻膠球，以震盪速率為 60 rpm 培養 60 min。將處理後

的藻膠球移入含有 1 mL 的 PVS2 溶液的冷凍管中，並置於液態氮中保存 1 h 後，取出冷凍管直接置入 40℃溫水中回溫 2 min，將冷凍管中的 PVS2 溶液吸出，再加入含 1.2 M 蔗糖 MS 基本鹽類培養液，靜置 15 min。最後，將藻膠球移至含 MS 基本鹽類培養基添加 1 μM NAA, 0.5 μM BA, 0.1 μM Kinetin, 30 g L^{-1} 蔗糖及 9.4 g L^{-1} Difco agar (Yu *et al*. 2012)或含 1 μM IAA, 1 μM BA, 30 g L^{-1} 蔗糖及 9.4 g L^{-1} Difco agar 的培養基，暗培養 7 d 後繼代培養一次，再置於光照 16 h，溫度 25 ℃條件下，培養 30 d 後，調查癒合組織形成率及植株成活率持續 90 d。

二、引子之設計

偵測 SPFMV、SPVG 及 SPCSV 等病毒的簡併式引子對係根據 GenBank 網站登錄的核苷酸序列資料比對設計，試驗中使用之引子對如 Table 1。

(一)RNA 之抽取及反轉錄-聚合酶連鎖反應

取 100 mg 的病葉，利用總量 RNA 純化試劑組(Plant Total RNA mini kit, Viogene, New Taipei, Taiwan)進行 RNA 之萃取，得到總量 RNA。以純化所得之總量 RNA 進行反轉錄-聚合酶連鎖反應 (reverse transcription-ploymerase chain reaction; RT-PCR)，RT-PCR 以單一步驟 RT-PCR 試劑組(Genemark Co., Taichung, Taiwan)進行，25 uL 的反應液中加入 1 uL 的 RNA，各 2.5 μL 之 20 μM 簡併式或專一性引子(Table 1)。RT-PCR 之進行於於熱循環反應儀(GeneAmp model 2400, Perkin-Elmer Co., Norwalk, CT, USA)中，設定反應程序為 50 ℃下進行反轉錄 30 min，94℃變性 1.5 min 後，進行 30 個 PCR 循環反應：94℃下變性 1 min，鍊合 1.5 min，溫度視引子序列而定，72℃下聚合 2 min，最後一個循環之 72℃聚合反應延長為 6 min。反應產物以 1.2%電泳瓊膠 (SeaKem, Agarose, Cambrex Bio Science Rockland, Inc., Rockland, ME, USA)進行分析。

(二)西方漬染法(Western blotting)

取 0.1 g 供試之病葉組織，以液態氮磨碎後，加入 200 μL 之樣品處理液(75 mM Tris-HCl, 10 mM KCl, 10 mM MgCl2, 1mM EDTA, 30% glycerol, 6% SDS, 9% β-Mercaptoethanol, 0.015% Bromophenol blue)，研磨液加熱處理後經 SDS-PAGE 電泳，膠體上之蛋白轉漬於 PVDF 膜(Millipore Co., MA, USA)，再以病毒抗血清進行漬染反應。甘藷病毒 G 抗血清稀釋 1000 倍進行反應，甘藷黃矮病毒抗血清稀釋 250 倍進行反應。

三、資料統計與分析

本試驗採完全逢機設計 (complete randomized design; CRD)，處理為 4 種不同品種(系)及 2 種不同培養基，每一處理重複 3 次以上，每一重複至少培養 4 個培植體，所獲得的資料進行每一品種(系)於兩種培養基的培養效果，利用最小顯著性差異(least signification difference test; LSD)比較各處理平均值間之差異。

Table 1. Sequences of primers used in this study and their amplicon size.

Name	Polarity	Nucleotide sequence	Amplicon size
SPFup	Sense	CCAAAYAT(A/C)AATGG(A/T)G(A/T)(T/G)TGGAC	200 bp
SPFGdw	Antisense	GCATATCGCGCAA(T/G)ACTCAT(A/G)TC	
SPGup	Sense	GA(T/C)TA(T/C)GG(A/T)GTGAA(T/C)GATGAGCA	280 bp
SPFGdwZ	Antisense	GCATATCGCGCAA(T/G)ACTCAT(A/G)TC	
SPCSup	Sense	AAATCT(T/C)GAGAAT(A/G)TGTT(T/C)ATGGT	350 bp
SPCSdw	Antisense	TCAACAGTGAAGACC(A/T)GT(A/T)CC(A/G)G	

Z In RT-PCR, both SPVFup and SPVGup as upstream primers and SPFGdw as the downstream primer.

結果與討論

去除植物病毒病的方法不外乎熱處理及生長點培養等(Cooper and Walkey 1978, Walkey and Cooper 1975)，近年來許多學者發現利用植物莖頂組織經液態氮處理後存活的植株，其獲得無病毒苗的比率較切取生長點為高(Brison *et al*. 1997, Helliot *et al* . 2002, Wang *et al*. 2003, 2006)。因此，本研究亦利用莖頂組織超低溫冷凍法對甘藷病毒去除的效果進行探討，以甘藷栽培種「台農 57 號」、「台農 66 號」與野生種品系「WT248」及「WT427」等 4 品種(系)病毒株的莖頂組織為材料，經藻膠包埋及超低溫冷凍(-196℃)處理後，回復生長培養基乃依據 Yu *et al.* (2012)所發表即 MS 基本鹽類添加 1 μM NAA, 0.5 μM BA, 0.1 μM Kinetin, 30 g L^{-1}蔗糖及 9.4 g L^{-1} Difco agar (A 培養基)，另一 B 培養基即 MS 基本鹽類添加 1 μM IAA, 1 μM BA, 0.1 μM Kinetin, 30 g L^{-1}蔗糖及 9.4 g L^{-1} Difco agar。以同一品種於 A 及 B 培養基分析結果，如 Table 2 顯示，4 個品種(系)的莖頂組織於 B 培養基所誘導的癒合組織形成率皆極顯著高於 A 培養基，其中以兩個野生種的癒合組織形成率更高達 93.5–94.8%；若將回復生長培養基中以 1 μM IAA 替代 NAA，並且將 BA 由 0.5 μM 提高為 1 μM (B medium)，則植株成活效果以「台農 57 號」及「台農 66 號」皆極顯著高於 A 培養基。其中，以「台農 57 號」效果最佳達 44.6% (Fig. 1)， 其次為「台農 66 號」的 15.6%，而「WT427」及「WT248」兩品系的植株形成率僅 0–11.1%；相反地，兩野生種品系於 A 培養基的植株形成率較 B 培養基達極顯著差異，其中以「WT427」的植株形成率最高達 15.2% (Fig. 2)。根據 Yu *et al.* (2012)以甘藷「台農 57 號」、「台農 66 號」、「台農 71 號」及「台農 73 號」莖頂組織為材料，以藻膠包埋玻璃質化法(Encapsulation–vitrification)進行超低溫冷凍保存，結果 4 個品種的植株成活率皆為 3.3%。本研究回復生長培養基先採用 Yu *et al.* (2012)所發表者，結果顯示於 4 種不同品種(系)經超低溫冷凍處後的植株成活率介於 2.2–15.2%，但將回復生長培養基中 BA 濃度提高 1 倍，除了野生種品系無效果外，極顯著提高栽培種的植株成活率；尤其「台農 57 號」提高 19.3 倍之多，「台農 66 號」增加 0.19 倍。而莖頂組織切取的大小亦為關鍵，Yu *et al.* (2012)所取的甘藷莖頂大小約 2–3 mm，經超低溫冷凍後植株成活率僅達 3.3%。Wang and Valkonen (2008)亦指出甘藷莖頂組織切太小約 0.5 mm 或太大約 1.5 mm 以上，進行超低溫冷凍處理後不利於植

Table 2. Effects of induced callus and regrowth of survived shoot tips for different medium and varieties (lines) of sweet potato after cryopreservation by encapsulation–vitrification.

	A medium[z]			B medium[y]		
Genotype	Explant No.	Callus (%)	Regrowth of survived shoot tips (%)	Explant No.	Callus (%)	Regrowth of survived shoot tips (%)
TNG57	104	70.0b[x]	2.2b	38	81.8a	44.6a
TNG66	55	84.7b	13.1b	14	88.9a	15.6a
WT248	104	89.7b	2.7a	46	93.5a	0.0b
WT427	51	85.2b	15.2a	35	94.8a	11.1b

[z] A media : full strength MS basal medium with 1 μM NAA, 0.5 μM BA, 0.1 μM Kinetin, 30 g L^{-1} sucrose and 9.4 g L^{-1} Difco agar at pH 5.7 ± 0.1. Data were collected after 90 d of culture.

[y] B media : full strength MS basal medium with 1 μM IAA, 1 μM BA, 30 g L^{-1} sucrose and 9.4 g L^{-1} Difco agar at pH 5.7 ± 0.1. Data were collected after 90 d of culture.

[x] Means within each column followed by the same letter(s) are not significantly (p < 0.01) different according to LSD test.

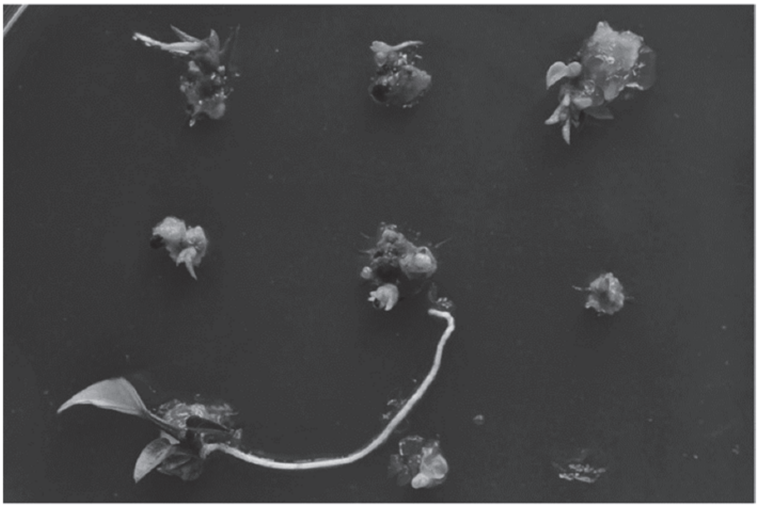

Fig. 1. The regrowth of survived shoot tips of 'TNG57' cultured on B medium after cryopreservation by encapsulation–vitrification for 60 d.

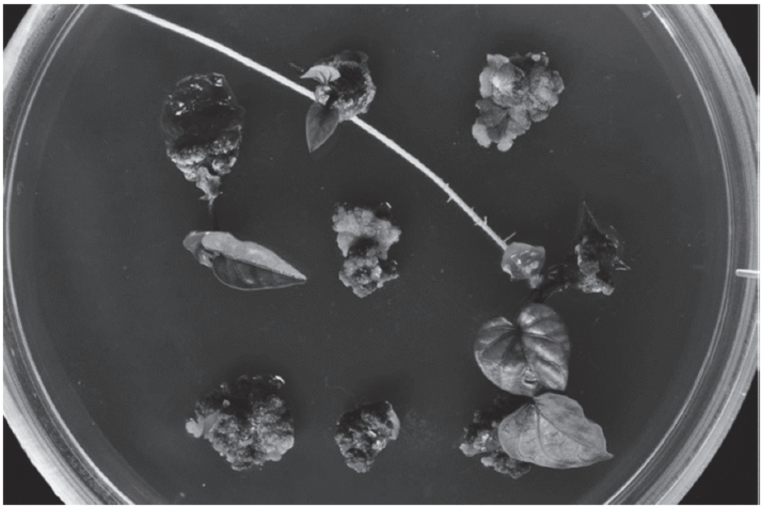

Fig. 2. The regrowth of survived shoot tips cultured on A medium after cryopreservation by encapsulation–vitrification on 'WT427' line for 60 d.

株成活，因此切含 3–4 片葉原基(約 1 mm)較有利於植株成活(Hirai and Sakai 2003)。本研究所取的甘藷莖頂大小亦為 1 mm，經超低溫冷凍處理後使栽培種的植株成活率大幅提高，但不同品種(系)之間所適合的回復生長培養基亦不同，並且可知癒合組織形成率的高低與植株形成率無關。

將甘藷「台農 57 號」、「台農 66 號」、「WT248」及「WT427」等 4 品種(系)有病毒的莖頂組織，經藻膠包埋超低溫處理後所存活的植株進行病毒病檢測，以 Table 1 的簡併式引子進行 RT-PCR，感染甘藷羽狀斑駁病毒增幅 200 bp 產物、感染甘藷病毒 G 增幅 280 bp 產物、感染甘藷黃矮病毒增幅 350 bp 產物。檢測供試甘藷材料，測出栽培種「台農 57 號」感染甘藷病毒 G 與甘藷黃矮病毒，「台農 66 號」感染甘藷羽狀斑駁病毒、甘藷病毒 G 與甘藷黃矮病毒，野生種「WT248」感染甘藷羽狀斑駁病毒與甘藷病毒 G、「WT427」感染甘藷病毒 G (Fig. 3)。4 種供

試品種亦以甘藷潛隱病毒(SPLV)的簡併式引子對進行 RT-PCR，但未測到此病毒(data not shown)，因此進行藻膠包埋超低溫處理後的再生植株不再偵測甘藷潛隱病毒。進行超低溫冷凍處理後所獲得的成活植株取下位葉進行病毒病檢測，即栽培種「台農 57 號」測試 13 再生植株、「台農 66 號」測試 6 再生植株，以 RT-PCR 均未測到上述三種病毒，去病毒率為 100% (Fig. 4)。「WT248」測試的 3 個再生植株中，都未測到病毒，去病毒率為 100%；野生種「WT427」測試的 9 個再生植株中有 5 個測到甘藷病毒 G 與 4 個未測到病毒，去病毒率為 44.4% (Fig. 5)，進行病毒病檢測之 4 品種再生植株的檢測結果如 Table 3。再生植株以西方漬染法偵測是否去病毒，任選 4 個‘TNG57’再生植株(‘TNG57’-1~4)，3 個‘TNG66’再生植株(‘TNG66’-1~3)，1 個‘T427’再生植株(‘WT427’-1)，分別以甘藷病毒 G 與甘藷黃矮病毒抗血清進行測試，結果只於作為對照的去病毒試驗前的‘TNG57’供

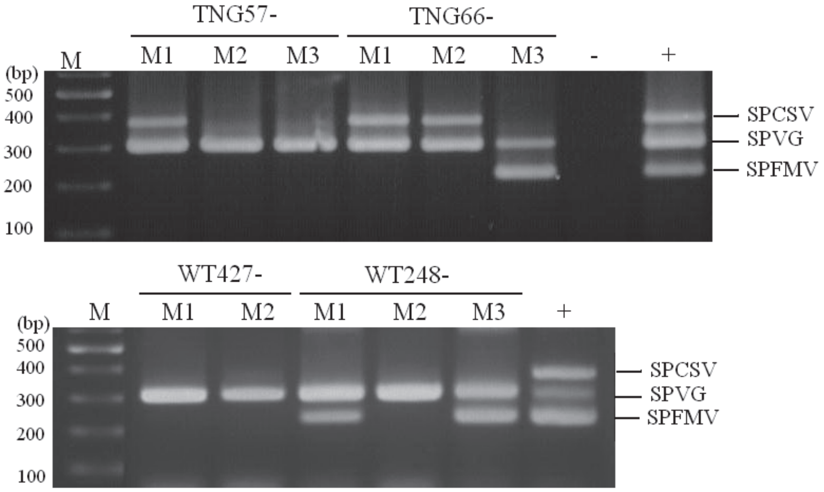

Fig. 3. Agarose gel analysis showing the amplification products obtained by RT-PCR for detection viruses from 4 varieties used in cryopreservation. Total RNA of healthy and 3 viruses infected ‘Tainung66’ (Lane – , +) was used as negative and positive control. Lane M: molecular size markers.

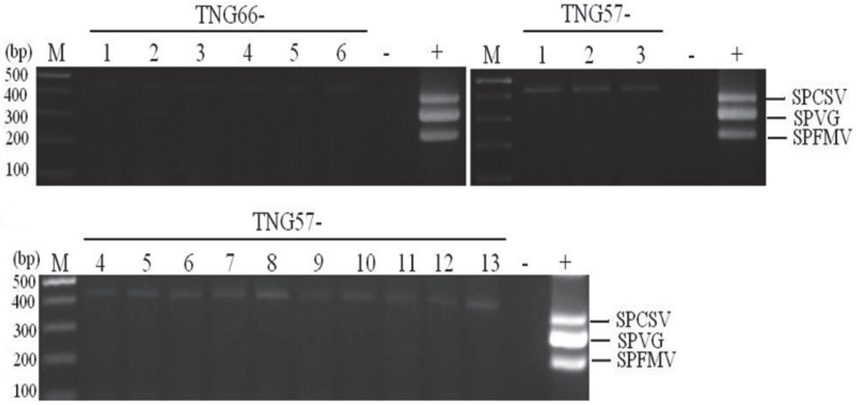

Fig. 4. Agarose gel analysis showing the amplification products obtained by RT-PCR for detection viruses in plantlets of ‘TNG57’ and ‘TNG66’ obtained from cryopreservation by encapsulation–vitrification. Total RNA of healthy and 3 viruses infected ‘TNG66’ (Lane – , +) was used as negative and positive control. Lane M: molecular size markers.

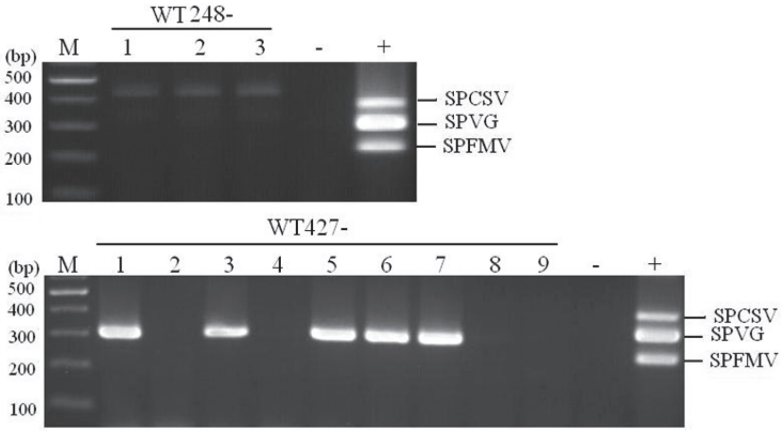

Fig. 5. Agarose gel analysis showing the amplification products obtained by RT-PCR for detection viruses in plantlets of 'WT427' and 'WT248' obtained from cryopreservation by encapsulation–vitrification. Total RNA of healthy and 3 viruses infected 'TNG66' (Lane – , +) was used as negative and positive control. Lane M: molecular size markers.

Table 3. Effects of virus elimination in plantlets of 4 varieties obtained from cryopreservation by encapsulation–vitrification.

Genotype (virus elimination ratio)		SPFMV	SPVG	SPCSV
TNG57 (100%)	1	ND	ND	ND
	2	ND	ND	ND
	3	ND	ND	ND
	4	ND	ND	ND
	5	ND	ND	ND
	6	ND	ND	ND
	7	ND	ND	ND
	8	ND	ND	ND
	9	ND	ND	ND
	10	ND	ND	ND
	11	ND	ND	ND
	12	ND	ND	ND
	13	ND	ND	ND
TNG66 (100%)	1	ND	ND	ND
	2	ND	ND	ND
	3	ND	ND	ND
	4	ND	ND	ND
	5	ND	ND	ND
	6	ND	ND	ND
WT-248 (100%)	1	ND	ND	ND
	2	ND	ND	ND
	3	ND	ND	ND
	1	ND	+	ND
	2	ND	ND	ND
WT427 (44.4%)	3	ND	+	ND
	4	ND	ND	ND
	5	ND	+	ND
	6	ND	+	ND
	7	ND	+	ND
	8	ND	ND	ND
	9	ND	ND	ND

試材料分別測到甘藷病毒 G 與甘藷黃矮病毒，'TNG57'與'TNG66'再生植株未測到 2 病毒，'WT427-1'雖於 RT-PCR 測到甘藷病毒 G，但在西方漬染法未測到此病毒(Fig. 6)，此結果與前人研究相符。甘藷羽狀斑駁病毒與甘藷黃矮病毒複合感染時，2 種病毒濃度都增高，才易以抗血清偵測，單獨感染時病毒濃度低，不容易利用抗血清偵測，但若以 RT-PCR 偵測較無此限制。

利用藻膠包埋玻璃質化法進行超低溫冷凍保存去病毒，已有香蕉等多個成功的例子(Wang and Valkonen 2008b)，甘藷亦有用此方法成功去除甘藷黃矮病毒與甘藷羽狀斑駁病毒(Wang and Valkonen 2008a)。本試驗以此法在 4 個供試品種都成功地去除病毒。栽培品種「台農 57」與「台農 66 號」供試材料各感染 2 或 3 病毒，且甘藷黃矮病毒與甘藷羽狀斑駁病毒或甘藷病毒 G 複合感染大幅提高病毒濃度(Karyeija *et al*. 2000)，但 2 栽培品種再生株經檢測後去病毒率 100%，反而單獨感染甘藷病毒 G 的野生種「WT427」去病毒率只有 44.4%，是否因試驗操作誤差，需要再重複試驗獲得更多再生株來釐清。Li *et al*. (2012)利用複合式 RT-PCR 同時偵測甘藷羽狀斑駁病毒、甘藷病毒 C、甘藷病毒 G 和甘藷病毒 Y。本試驗中合併甘藷羽狀斑駁病毒、甘藷病毒 G 和甘藷黃矮病毒於同一 RT-PCR，亦可大幅減少 RT-PCR 測試次數。但在「台農 57 號」與野生種「WT248」的產物電泳分析會有一 400 bp 微弱 DNA 條帶，RT-PCR 單獨檢測 3 病毒時未見此條帶，經定序分析後為甘藷基因之非專一性反應。

Benson (2008)指出植物超低溫冷凍時脫水及玻璃質化處理，使植物對低溫有忍受性，可防止冰晶產生使器官及細胞膜減緩傷害，因此提高細胞的存活。Helliot *et al*. (2001)說明植物無論在冷凍或解凍階段所產生的傷害，最主要為細胞內產生的結晶水，所以可以存活的小範圍細胞僅在葉原基及生長點的位置(Haskins and Kartha 1980)。另外，超低溫冷凍處理後快速的高溫處理，使病毒 RNA 的數量降低許多(Wang *et al*. 2008)。綜合以上的結論，可知含有病毒的甘藷莖頂組織於進行藻膠包埋時會經脫水及玻璃質化處理後，再放入液態氮(-196℃)處理 1h 後，以 40℃水溫快速回溫 2 min，置入回復生長的培養基，因此使成活的植株能獲得高比率的健康株(Table 3)，此結果使甘藷種原除了能長期保存且為健康的種原具有深遠的意義。

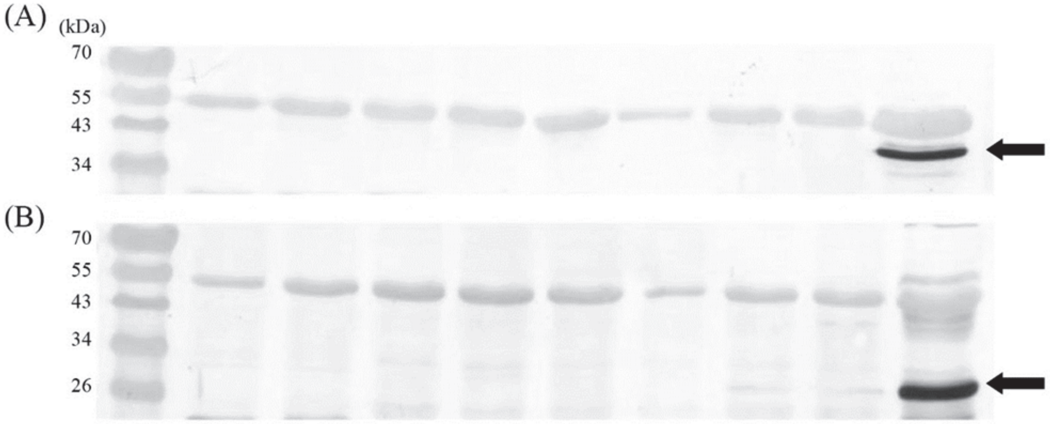

Fig. 6. Western blotting analysis of SPVG (A) and SPCSV (B) from plantlets of obtained from cryopreservation by encapsulation–vitrification. Lanes 1-4: plantlets of 'TNG57'-1~4; Lanes 5-7: plantlets of 'TNG66'-1~3; Lane 8: plantlets of 'WT427'-1; Lane +: virus infected 'TNG57'; and Lane M: protein markers.

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